Leucine-Rich Repeat Extensin Proteins and Their Role in Cell Wall Sensing

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Abstract: Plant cells are surrounded by a cell wall that provides shape and physically limits cell expansion. To sense the environment and status of cell wall structures, plants have evolved cell wall integrity-sensing mechanisms that involve a number of receptors at the plasma membrane. These receptors can bind cell wall components and/or hormones to coordinate processes in the cell wall and the cytoplasm. This review focuses on the role of leucine-rich repeat extensins (LRXs) during cell wall development. LRXs are chimeric proteins that insolubilize in the cell wall and form protein–protein interaction platforms. LRXs bind RALF peptide hormones that modify cell wall expansion and also directly interact with the transmembrane receptor FERONIA, which is involved in cell growth regulation. LRX proteins, therefore, also represent a link between the cell wall and plasma membrane, perceiving extracellular signals and indirectly relaying this information to the cytoplasm.

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Leucine-Rich repeat eXtensin (LRX) Proteins and their role in cell wall sensing

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Summary

Plant cells are surrounded by a cell wall which provides shape and physically limits cell expansion. In order to sense the environment and status of cell wall structures, plants have evolved cell wall integrity sensing mechanisms which involve a number of receptors at the plasma membrane. These receptors can bind cell wall components and/or hormones to coordinate processes in the cell wall and the cytoplasm. This review focuses on the role of Leucine Rich Repeat (LRR)-extensins (LRXs) during cell wall development. LRXs are chimeric proteins that insolubilize in the cell wall and form protein-protein interaction platforms. LRXs bind RALF peptide hormones that modify cell wall expansion and also directly interact with the transmembrane receptor FERONIA, which is involved in cell growth regulation. LRX proteins, therefore, also represent a link between the cell wall and plasma membrane, perceiving extracellular signals and indirectly relaying this information to the cytoplasm.

Introduction

Unlike animal cells which have no cell wall, expansion of plant cells requires a unique set of regulatory mechanisms in order to modify the extracellular matrix. The cell wall provides protection against biotic and abiotic stresses and, importantly, defines the shape of each cell. The primary cell wall of expanding cells is composed of a number of polysaccharides that are classified into cellulose, hemicelluloses, and pectins. Cellulose and hemicellulose undergo interactions and this network is embedded in gel-like pectins [1]. In addition to polysaccharides, the presence of structural cell wall proteins, characterized by highly repetitive sequence motifs, can modify mechanical properties of the cell wall. These proteins are particularly rich in specific amino acids and are therefore classified as glycine-rich, proline-rich, or hydroxyproline-rich glycoproteins [2-4]. The cell interior (protoplast) exerts turgor pressure as the driving force for cell growth, which is counteracted by the surrounding cell wall. To facilitate cellular expansion, remodeling of cell wall structures, degradation of cell wall components and the biosynthesis and incorporation of new material into the expanding cell wall must occur. Thus, cell wall remodeling requires a large number of proteins with diverse biochemical activities [5]. An elaborate cell wall integrity (CWI) surveillance system consisting of apoplastic proteins and transmembrane receptors detects changes in cell wall homeostasis. It can induce changes in turgor pressure or elicit compensatory modifications in cell wall structures [6-8]. CWI sensing involves a number of transmembrane receptor kinases at the plasma membrane that can bind cell wall components and trigger intracellular processes. Among these are wall-associated kinases, LRR-receptor kinases and *Catharanthus roseus* receptor kinase-like 1 like (*Cr*RLK1L) proteins [9-12]. This review focuses on the role of extracellular Leucine-rich Repeat eXtensin

(LRX) proteins in the exchange of signals between intra- and extracellular compartments, coordinating processes on both sides of the plasma membrane during cell growth.

Extensins

Extensins are a group of hydroxyproline-rich glycoproteins structural cell wall proteins characterized by Ser-Pro_{2-n} repeats [13, 14]. The Pro residues in this repetitive context are posttranslationally hydroxylated by Proline-hydroxylases (P4Hs) [15] that recognize contiguous Pro residues preceded by a Ser [16], resulting in Ser-Hyp_{2-n} repeats. The contiguous Hyps in extensins can be O-glycosylated with chains up to 4-5 linear arabinoses on each Hyp by the activity of a series of arabinosyltransferases [17-20]. The Ser residues are monogalactosylated by a galactosyltransferase encoded by a single gene in *Arabidopsis* [21]. Extensins form polyproline II helices and the polysaccharide sidechains are thought to wrap around the protein to stabilize the structure in a similar way to collagens in animals which form trimeric helices [22]. Intra- and intermolecular linkages by oxidative crosslinking of Tyr in the context of Tyr-X-Tyr sequence resulting in isodityrosines, di- isodityrosines, or pulcherosines [14, 23-26] further strengthen the extensin structure.

Extensins are an essential component of the cell wall. The embryo lethal extensin mutant *rsh1* fails to form properly connected cell walls during cell division. RSH1 was shown to be released early during cell division, forming a self-aggregating initial scaffold to which pectins can adhere, resulting in new cell walls separating the daughter cells [27, 28]. In response to pathogens, extensin networks can modify mechanical properties of cell walls, hindering or delaying pathogen invasion [29-31].

Chimeric extensin proteins are candidates for having a regulatory or signaling function since the extensin domain can interact with the cell wall while the second, non-structural domain provides an independent activity [32]. Proline-rich extensin receptor kinases (PERKs) are involved in cellular growth control and have an extracellular domain with strong homology to extensins while the cytoplasmic domain shows kinase activity [33]. Formin-homolog extensins are transmembrane proteins with a cytoplasmic actin-binding domain and an extracellular extensin domain, indicative of a direct influence of the cell wall on actin dynamics [34-36].

LRR-Extensins

Leucine-rich repeat (LRR)-extensins (LRXs) are cell wall-localized chimeric extensin proteins. The signal peptide for protein export is followed by an N-terminal (NT) domain of around 70

amino acids. The first half of this domain is variable among LRXs and even partly missing in LRX1, LRX2, LRX6, and LRX7, and is followed by a well-conserved sequence of around 34 amino acids (Figure 1). There is no clear homology of the NT-domain to sequences of known function in other proteins except predicted LRR-extensins, suggesting an LRX-specific function for this domain. The LRR domain contains 11 leucine-rich repeats. This motif is found in organisms of all kingdoms and represents a versatile domain implicated in binding interaction partners that range from small peptides to large proteins and non-proteinaceous small molecular compounds such as brassinosteroids [37]. The very recent elucidation of the crystal structure of the LRR domain of LRXs revealed covalent dimerization by a disulfide bond involving a conserved Cys residue in the LRR domain and N-glycosylation at several positions [38]. A Cys-rich domain (CRD) of 39-50 amino acids separates the LRR- and extensin domains (Figure 1). According to the crystal structure, the Cys residues in this region form disulfide bonds with Cys residues of the LRR domain and may act to stabilize the structure of the LRX protein [38]. As for the NT-domain, a BLAST search with the CRD identifies exclusively predicted LRR-extensins of other plant species, suggesting that it could be specifically required for LRX function.

The extensin domain of LRXs is frequently composed of several distinct sequence motifs that are repeated several times, but can also be very short (Figure 2). In general, the extensin domain is highly variable among LRXs in terms of length and amino acid sequence, making protein alignment of the extensin domains difficult. Deletion analysis of the LRX1 extensin domain revealed that a large part including motif II and motif III (Figure 2), can be removed without apparent loss of protein activity [39]. Next to Pro and Ser, the extensin domain is rich in Tyr and Val. As described above, Tyr can be important for crosslinking of EXTs and insolubilization in the cell wall [40]. However, the insolubilization of LRX1 in the cell wall does not depend on these Tyr residues [39], suggesting that glycosides linked to the extensin might be involved in crosslinking of the protein [41] and that Tyr might be required for establishing the correct tertiary structure of the protein. There is currently no evidence that the modification of cell wall properties by crosslinking of the extensin domain would be an intrinsic function of LRXs. Overexpression of the *LRX1* extensin coding sequence does not alter the *lrx1* root hair defect [39]. Hence, the extensin domain of LRXs likely serves an anchoring function to position and immobilize the protein in the cell wall.

LRX gene family

LRX genes of higher plants can be classified into genes predominantly expressed in vegetative tissue and in pollen grains/pollen tubes. The pollen-localized LRXs of different species are more homologous to each other than to LRX proteins of the same plant species that are expressed in vegetative tissues [32, 42]. This suggests that pollen-expressed *LRXs* may have

functions that are specific to pollen or pollen tubes. The family of *LRX* genes in *Arabidopsis thaliana* encompasses eleven members (*LRX1-LRX11*). Gene expression studies revealed a group (*LRX1-LRX7*) that are expressed in vegetative tissue, and a group of four pollen-expressed (*LRX8-LRX11) genes* [42]. The latter were initially named *PEX1* (*pollen-expressed LRX 1*)-*PEX4*, but later renamed *LRX8-LRX11* [43] to avoid confusion with the Arabidopsis *PEX* genes that are involved in the biogenesis and maintenance of peroxisomes [44].

Among the vegetatively expressed *LRXs*, *LRX1* and *LRX2* are predominantly expressed in root hairs. An *lrx1* single mutant develops deformed root hairs that frequently burst [45]. The *lrx2* single mutant bears wild type-like root hairs, but the *lrx1 lrx2* double mutant is more severely impaired in root hair development than *lrx1*, suggesting synergistic interaction and very similar activity of LRX1 and LRX2. Cell walls of *lrx1 lrx2* root hairs are less dense in structure, possibly weakening their mechanical stability, which would explain their frequent bursting [46]. The expression of *LRX2*, is not completely restricted to root hairs as it has also been shown to be involved in lateral root formation. The details on the activity of LRX2 in this process is not yet understood [47]. *LRX3-LRX5* are expressed in roots (but not root hairs) and the shoot. Corresponding single mutants do not display obvious phenotypes. However, the *lrx3lrx4lrx5* triple mutant shows stunted growth, broader rosette leaves with cell-cell adhesion defects in the epidermal cell layer, a cell and vacuolar growth defect, salt-hypersensitivity, as well as increased anthocyanin accumulation [48-50]. Only subtle changes in cell wall structures were identified in this mutant, indicating that mutations in these *LRX*s have several rather mild, but cumulating defects in cell wall formation. *LRX6* and *LRX7* are expressed during lateral root formation and flower development, respectively [42], but await functional characterization. *LRX8-LRX11* are mainly expressed in pollen and higher-order mutants of these genes develop defects in cell wall formation in pollen grains and tubes. These mutants also over-accumulate callose, a glucose polymer mainly found in pollen [51], presumably to support the weakened cell wall. Quadruple mutants of *lrx8-11* have defects in pollen tube growth including frequent bursting, resulting in strongly reduced fertility [43, 52, 53]. Ca^{2+} homeostasis is also affected in these *lrx* mutants and the growth phenotype can be alleviated by reducing Ca^{2+} uptake. This suggests a role of LRXs in regulating Ca2+ dynamics [43].

LRX-RALF interactions

The LRR domains of LRX proteins were recently identified as high-affinity binding sites of RALFs (rapid alkalinization factors) [49, 50, 54, 55]. RAFLs are peptide hormones which modify plant growth, fertilization, and responses to pathogen infection by inducing several physiological responses, including alkalinization of the apoplast [56-60]. The acid growth hypothesis suggests that acidification of the cell wall favors cell wall expansion, whereas

alkalinization causes arrest of elongation [5, 61]. The Arabidopsis *RALF* gene family contains 34 members, some of which are broadly expressed, while others are found in specific tissues [56, 57]. Several lines of evidence demonstrate that LRX proteins interact with RALFs. Pollen tubes of plants with mutations in three or more of the pollen-expressed *LRX8-LRX11* were shown to be insensitive to the growth arresting effect of pollen-expressed RALF4 and showed reduced binding of RALF4 to the pollen tube surface. The results of yeast two-hybrid assays, co-immunoprecipitation and biolayer interferometry experiments further support that a direct interaction between pollen expressed LRXs and RALF4 occurs [54]. Crystallization of the LRX-RALF complex revealed that an LRX dimer binds two RALF peptides, exposing a highly basic surface patch of RALF which could facilitate interactions with other protein or cell wall components [38]. Taken together, analyses of the *lrx8-lrx11* mutants provide strong evidence that RALF4 requires pollen-expressed LRXs to maintain cell wall integrity during pollen tube growth [54]. RALF1 is found in roots and the shoot where it has strong effects on plant growth [62-64]. The root/shoot-expressed LRX4 was subsequently found to bind RALF1 [49], confirming that LRX proteins bind RALFs in different tissues. Immuno-precipitation of LRX3, LRX4, and LRX5 resulted in co-purification of an overlapping but not identical range of RALF peptides [50]. Hence, RALF-LRX interactions seem to include a number of possible combinations. This could potentially be a way to provide specificity to defined biological activities of the large number of RALF peptides present in the plant. Certainly, not all LRXs are high-affinity binding sites for all RALFs. For example, LRX8 binds RALF1 *in vitro* with a 1000-fold reduced affinity compared to RALF4 [54].

LRXs-CrRLK1L receptor interactions

The implication of LRXs in RALF-dependent signaling processes suggests a link to *Cr*RLK1Ls, which are *bona fide* receptors for RALF peptides [60, 62, 65]. *Cr*RLK1Ls are transmembrane proteins with two extracellular malectin-like domains and a cytoplasmic kinase domain. This protein structure predetermines the family of 17 members in *Arabidopsis thaliana* to transduce cell wall-derived signals and trigger intracellular responses [11, 12, 66]. The first evidence for a role of *Cr*RLK1Ls in governing cell wall-related processes was the description of a cell wall integrity sensing function for *Cr*RLK1LTHESEUS1 (THE1). The *the1* mutation mitigates the dark-grown hypocotyl elongation defects of various cellulose deficient mutants. The absence of a strong phenotypic effect in *THE1* loss- and gain-of-function lines in the absence of cellulose-deficiency illustrated that THE1 monitors cell wall composition and actively represses growth in cellulose-deficient mutants [6, 67]. The *Cr*RLK1L FERONIA (FER) has been linked to a cell wall surveillance system that, controls disintegration of the pollen tube cell wall during pollen tube perception at the synergid cells during fertilization [68] and monitors cell wall

integrity during salt stress [69]. FER mutants show altered Ca2+ signals, which is linked to changes in the perception of mechanical stimulations [70, 71]. Similarly, the *Cr*RLK1Ls ANXUR (ANX)1/2 and BUDDHA'S PAPER SEAL (BUPS)1/2 are involved in cell wall integrity maintenance during pollen tube growth prior to reaching ovules [60, 72]. Mutations in another *Cr*RLK1L gene, *ERULUS,* leads to modified Ca2+ fluxes resulting in aberrant root hair formation [73, 74]. FERONIA has been shown to be the receptor for RALF1, and several *Cr*RLK1L proteins bind different RALF peptides with varying specificities [59, 60, 62, 65].

The *lrx3lrx4lrx5* triple mutant shares some phenotypic aspects of the *fer-4* mutant including stunted growth and salt stress responses, which suggests that they are active in the same pathway [49, 50]. Employing co-immunoprecipitation and yeast two-hybrid assays, a physical link between FER and the LRR domain of LRX4 was demonstrated [49]. We show that LRX/FER-dependent cell wall sensing is required to coordinate extra- and intracellular adaptations. Vacuolar morphology is influenced by the extracellular pH and this adjustment depends on both FER and LRXs function [49]. LRXs constitute a physical link between the plasma membrane (via the association of the LRR domain with FER) and the extracellular matrix (via the extensin domain). A membrane association of LRXs lacking the extensin domain supports this hypothesis [43]. In addition to its growth-coordinating activity, the LRX-FER axis is also important for CWI sensing. Applying the pectin modifying compound epigallocatechin gallate induces alterations in the cell wall that result in changes in vacuolar morphology. This response is FER and LRX dependent, with mutations in these genes preventing proper response to EGCG treatment or substrate stiffening [49].

It should be noted that in addition to its extracellular interaction with LRX, FER has been shown to collaborate with other membrane-associated proteins, thereby regulating a wide range of biological processes. For instance, FER interacts, in a RALF23-dependent fashion, with BRI1-associated Kinase 1 (BAK1)/somatic embryogenesis receptor Kinase 3 (SERK3). The (flg22) ligand-induced FER/BAK1 complex serves a scaffolding function for the recruitment of the EF-TU RECEPTOR (EFR) and FLAGELLIN-SENSING 2 (FLS2) receptors during plant immune responses [59]. Unlike *fer* and *lrx* mutants, vacuolar morphology of *bak1* mutants is unaffected in comparison to the wild type, implying that the FER/LRX-dependent cell wall sensing mechanism is distinct from the receptor scaffolding mechanism [75]. FERONIA has also been shown to interact with the glycosylphosphatidylinositol-anchored proteins LORELEI (LRE) and LRE-LIKE GPI-AP1 (LLG1) in the ER and this interaction is crucial for the localization of FER to the plasma membrane, implying that LRE and LLG1 act as chaperones for FER throughout the secretory pathway [76]. The pleiotropic phenotypes of the *fer* mutant point to FER being a central player as a scaffold protein with a relay function in multiple processes. Future analyses will have to reveal the extent to which LRX proteins influence the different functions of FER and whether they also interact with FER-interacting proteins.

Dynamics of LRX-RALF-*Cr*RLK1L interactions

The potential for LRX-RALF, *Cr*RLK1L-RALF and *Cr*RLK1L-LRX interactions would allow numerous ways for the plant to regulate cell-wall monitoring systems and cell-wall modification. In pollen tubes, ANX/BUPS heteromers as well as LRX8/LRX9/LRX10/LRX11 bind RALF4/19 [54, 60]. It is unclear whether these interactions are independent or whether RALF4/19 binding induces the formation of a ANX/BUPS/LRX/RALF multiprotein complex (Figure 3). The premature pollen tube bursting phenotype of mutants affected in the pollen-expressed *LRXs* (*LRX8* - *LRX11*) is similar to *amiRRALF4/19* lines and loss-of-function mutants of *ANX1/ANX2,* suggesting that they could interact in the same complex or share the same regulatory pathway. Although LRX and FER interact with RALFs, LRX/FER interaction can take place in the absence of RALFs, suggesting that RALFs may not be required for all aspects of LRX-FER signaling [49] (Figure 4). Both LRX4 and FER can bind RALF1, but it remains to be shown whether these interactions have synergistic or antagonistic effects on the formation of the complex. The *lrx3lrx4lrx5* triple mutant roots show mildly reduced sensitivity, while *fer* mutant roots are strongly resistant to RALF1 [49, 62], indicating that LRX and FER are both active in triggering the RALF1-induced signaling process. Additional insights were obtained from experiments with LRXs missing the cell wall-anchoring extensin domain (LRXΔE). Expressing *LRX1ΔE* in root hairs of the wild type induces a root hair deformation reminiscent of the *lrx1*, *lrx1 lrx2*, and *fer* phenotypes that are characterized by aberrant root hair formation [39, 45, 46, 77]. Accordingly, the overexpression of *LRX4ΔE* in the wild type induces phenotypes comparable to the *lrx3lrx4lrx5* and *fer* mutants [49]. These data show that overexpression of *LRXΔE* constructs cause a dominant-negative effect, suggesting interference of LRX-RALF-FER activity. Extensins tightly interact with the cell wall [25, 78], and do so also in the context of LRXs [39, 45]. Thus, anchoring of LRXs is essential for their activity, suggesting that LRXs possibly locally restrict movement of the LRX-RALF-FER complex. Alternatively, a physical strain acquired by interacting with the cell wall and FER in the plasma membrane may be required for LRXs to be active. The crystal structure suggests that binding of RALF to the LRR region could affect the overall conformation of the LRX protein and possibly impact on the extensin domain that has not been included in the crystallization study [38].

LRX4ΔE overexpression lines induce *lrx*/*fer* mutant-like phenotypes, but cause RALF1 hypersensitivity in seedling root growth assays [49], revealing a high complexity of the LRX, RALF, and FER signaling. It is currently difficult to merge our understanding of LRXs into a coherent model given the multitude of interactions observed and their varying degrees of affinity. Different RALFs that are bound by FER and/or LRXs might influence complex formation in opposing ways [49, 50] and different LRXs show different protein binding dynamics

in the presence of RALF peptides (Figure 2). It is also possible that there are unknown factors involved in this process which remain to be identified in order to understand the diverse activities of LRXs and the LRX-RALF-FER/CrRLK1Ls network. The amount of available RALF peptides seem to have a strong impact on the *lrx* mutant phenotypes. A mutation in *S1P*, encoding the subtilase that processes pre-RALFs into the biologically active RALFs, suppresses the salt-sensitivity phenotype of the *lrx3lrx4lrx5* triple mutant [50]. A possible explanation of this observation is that in the absence of LRXs, the mutant phenotypes are at least in part induced by the increased availability of RALFs, an effect that is alleviated by interfering with RALF maturation. Thus, LRX proteins not only serve as RALF binding sites to induce certain processes. With the competence to bind RALFs, LRXs seem to control their abundance in the apoplast. Whether the RALF binding capacity of LRXs is influenced by transient posttranslational modifications of LRXs is yet another open question that needs to be investigated.

Genetic integrators of LRX function

In addition to a better understanding of the FER/LRX/RALF complex formation dynamics, the elucidation of genetic interaction partners will shed light on the molecular mechanisms related to the LRX signaling pathway. The easily scorable root hair deformation phenotype of the *lrx1* mutant made it ideal for a forward genetic screen to identify suppressors of *lrx1*, which are likely involved in LRX1-related activities. A number of *rol* (*repressor of lrx1*) mutants were identified, displaying wild type-like root hairs despite the presence of the *lrx1* mutation. The *ROL1* locus encodes the rhamnose-synthase RHM1. Rhamnose is an important component of the pectin rhamnogalacturonan I (RGI) and RGII, and the identified *rol1* mutants show an altered pectin structure and a reduction in RGII content [79]. FER is a CWI sensor with a shown ability to bind pectin fragments [69]. It is possible that the cell wall defects induced by the *rol1* mutation are perceived by FER, and compensatory changes in cell wall structures are induced resulting in suppression of the *lrx1* phenotypes. In contrast to the *rol1* mutants, *rol5* and *rol17* suppress the *lrx1* root hair defect in a very different way. *ROL5* encodes a cytoplasmic thiouridylase involved in thiolating tRNAs, whereas *ROL17* encodes a isopropylmalate synthase IPMS1 necessary for the biosynthesis of the amino acid leucine [80-83]. While the mode of action of these two *rol* mutants initially seemed rather enigmatic, more detailed analyses revealed that both affect TOR (Target of Rapamycin) signaling. The TOR network is a major controller of eukaryotic cell development that senses nutrient availability and growth factors, and modifies a number of processes related to cell growth including mitochondrial activity, ROS production, translation, and actin cytoskeleton dynamics [84]. Both *rol5* and *rol17* alter translational activity [80, 85-87]. This is likely to cause a feedback signal to the TOR

network, which adjusts growth through the activity of the TOR kinase, the central component of the TOR network [88, 89]. Application of specific inhibitors of the TOR kinase to roots results in reduced cell growth, alterations in cell wall structures, and, when applied to the *lrx1* mutant, suppression of the *lrx1* root hair phenotype [80, 81]. A potential connection between the TOR network and FER/LRX protein function might be provided by ROP2 (Rho-related GTPase of plants), a GTP binding protein that regulates cell growth by binding to and activating the TOR kinase in an auxin-dependent manner [90]. ROP2 is also a known signaling component of the FER-pathway regulating cell growth [77]. This suggests that one strategy of the TOR network to modify cell growth is through influencing FER-triggered signaling.

Conclusion

In conclusion, LRX proteins are cell wall-localized components of a system that transfers information from the cell wall to the cytoplasm in order to regulate and coordinate cell growth as well as cell wall formation. They do so as high-affinity binding sites for a number of RALF peptides and via interaction with the *Cr*RLK1L-type receptor kinase FER. It is conceivable that LRX proteins also interact with additional plasma membrane-localized proteins of the *Cr*RLK1L family and/or with other proteins. The particular chimeric structure of LRX proteins allows for a strong interaction with the cell wall, thus providing a direct physical link with transmembrane receptors. This also suggests a role for LRXs in the coordination of cell wall and protoplast expansion by sensing connection of the cell wall with the plasma membrane. Findings in recent years have provided highly interesting insights into LRX function. Future research will elucidate the dynamics of the different interaction networks, in order to better understand the activities of the different proteins in regulating the cell wall and cellular expansion.

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References

1. Cosgrove, D.J. (2014). Re-constructing our models of cellulose and primary cell wall assembly. Curr. Opin. Plant Biol. *22*, 122-131.
2. Showalter, A.M. (1993). Structure and function of plant cell wall proteins. Plant Cell *5*, 9-23.
3. Wu, H., De Graaf, B., Mariani, C., and Cheung, A.Y. (2001). Hydroxyproline-rich glycoproteins in plant reproductive tissues: structure, functions and regulation. Cell. Mol. Life Sci. *58*, 1418-1429.
4. Ringli, C., Keller, B., and Ryser, U. (2001). Glycine-rich proteins as structural components of plant cell walls. Cell. Mol. Life Sci. *58*, 1430-1441.
5. Cosgrove, D.J. (2014). Plant cell growth and elongation. eLS *DOI: 10.1002/9780470015902.a0001688.pub2*.
6. Hématy, K., Sado, P.-E., Van Tuinen, A., Rochange, S., Desnos, T., Balzergue, S., Pelletier, S., Renou, J.-P., and Höfte, H. (2007). A receptor-like kinase mediates the response of Arabidopsis cells to the inhibition of cellulose synthesis. Curr. Biol. *17*, 922-931.
7. Wolf, S., Hematy, K., and Hoefte, H. (2012). Growth control and cell wall signaling in plants. Annu. Rev. Plant Biol. *63*, 381-407.
8. Kohorn, B.D., Kobayashi, M., Johansen, S., Riese, J., Huang, L.F., Koch, K., Fu, S., Dotson, A., and Byers, N. (2006). An Arabidopsis cell wall-associated kinase required for invertase activity and cell growth. Plant J. *46*, 307-316.
9. Kohorn, B.D. (2016). Cell wall-associated kinases and pectin perception. J. Exp. Bot. *67*, 489-494.
10. Xu, S.L., Rahman, A., Baskin, T.I., and Kieber, J.J. (2008). Two leucine-rich repeat receptor kinases mediate signaling, linking cell wall biosynthesis and ACC synthase in Arabidopsis. Plant Cell *20*, 3065-3079.
11. Nissen, K.S., Willats, W.G.T., and Malinovsky, F.G. (2016). Understanding *Cr*RLK1L Function: cell walls and growth control. Trends Plant Sci. *21*, 516-527.
12. Franck, C.M., Westermann, J., and Boisson-Dernier, A. (2018). Plant malectin-like receptor kinases: from cell wall integrity to immunity and beyond. In Annu. Rev. Plant Biol., Volume 69, S.S. Merchant, ed., pp. 301-328.
13. Borassi, C., Sede, A.R., Mecchia, M.A., Salgado Salter, J.D., Marzol, E., Muschietti, J.P., and Estevez, J.M. (2016). An update on cell surface proteins containing extensin-motifs. J. Exp. Bot. *67*, 477-487.
14. Showalter, A.M., and Basu, D. (2016). Extensin and arabinogalactan-protein biosynthesis: glycosyltransferases, research challenges, and biosensors. Front. Plant Sci. *7*.
15. Velasquez, S.M., Ricardi, M.M., Poulsen, C.P., Oikawa, A., Dilokpimol, A., Halim, A., Mangano, S., Denita Juarez, S.P., Marzol, E., Salgado Salter, J.D., et al. (2015). Complex regulation of prolyl-4-hydroxylases impacts root hair expansion. Mol. Plant *8*, 734-746.
16. Canut, H., Albenne, C., and Jamet, E. (2016). Post-translational modifications of plant cell wall proteins and peptides: A survey from a proteomics point of view. Biochem. Biophys. Acta *8*, 983-990.
17. Egelund, J., Obel, N., Ulvskov, P., Geshi, N., Pauly, M., Bacic, A., and Petersen, B.L. (2007). Molecular characterization of two *Arabidopsis thaliana* glycosyltransferase mutants, *rra1* and *rra2*, which have a reduced residual arabinose content in a polymer tightly associated with the cellulosic wall residue. Plant Mol. Biol. *64*, 439-451.
18. Gille, S., Hänsel, U., Ziemann, M., and Pauly, M. (2009). Identification of plant cell wall mutants by means of a forward chemical genetic approach using hydrolases. Proc. Natl. Acad. Sci. U.S.A. *106*, 14699-14704.
19. Moller, S.R., Yi, X., Velasquez, S.M., Gille, S., Hansen, P.L., Poulsen, C.P., Olsen, C.E., Rejzek, M., Parsons, H., Yang, Z., et al. (2017). Identification and evolution of a plant cell wall specific glycoprotein glycosyl transferase, ExAD. Scientific Reports *7*.
20. Showalter, A.M., Keppler, B.D., Liu, X., Lichtenberg, J., and Welch, L.R. (2016). Bioinformatic Identification and Analysis of Hydroxyproline-Rich Glycoproteins in Populus trichocarpa. BMC Plant Biol. *16*, 229.

21. Saito, F., Suyama, A., Oka, T., Yoko-o, T., Matsuoka, K., Jigami, Y., and Shimma, Y. (2014). Identification of novel peptidyl serine α-galactosyltransferase gene family in plants. J. Biol. Chem. *289*, 20405-20420.
22. Cejas, M.A., Kinney, W.A., Chen, C., Leo, G.C., Tounge, B.A., Vinter, J.G., Joshi, P.P., and Maryanoff, B.E. (2007). Collagen-related peptides: Self-assembly of short, single strands into a functional biomaterial of micrometer scale. J. Am. Chem Soc. *129*, 2202-2203.
23. Fry, S.C. (1982). Isodityrosine, a new cross-linking amino acid from plant cell-wall glycoprotein. J. Biochem. *204*, 449-455.
24. Brady, J.D., Sadler, I.H., and Fry, S.C. (1996). Di-isodityrosine, a novel tetrameric derivative of tyrosine in plant cell wall proteins: A new potential cross-link. Biochem. J. *315*, 323-327.
25. Held, M.A., Tan, L., Kamyab, A., Hare, M., Shpak, E., and Kieliszewski, M.J. (2004). Di-isodityrosine is the intermolecular cross-link of isodityrosine-rich extensin analogs cross-linked in vitro. J. Biol. Chem. *279*, 55474-55482.
26. van Holst, G.J., and Varner, J.E. (1984). Reinforced polyproline ii conformation in a hydroxyproline-rich cell wall glycoprotein from carrot root. Plant Physiol. *74*, 247-251.
27. Cannon, M.C., Terneus, K., Hall, Q., Tan, L., Wang, Y.M., Wegenhart, B.L., Chen, L.W., Lamport, D.T.A., Chen, Y.N., and Kieliszewski, M.J. (2008). Self-assembly of the plant cell wall requires an extensin scaffold. Proc. Natl. Acad. Sci. U.S.A. *105*, 2226-2231.
28. Hall, Q., and Cannon, M.C. (2002). The cell wall hydroxyproline-rich glycoprotein RSH is essential for normal embryo development in Arabidopsis. Plant Cell *14*, 1161-1172.
29. Brady, J.D., and Fry, S.C. (1997). Formation of di-isodityrosine and loss of isodityrosine in the cell walls of tomato cell-suspension cultures treated with fungal elicitors or H2O2. Plant Physiol. *115*, 87-92.
30. Castilleux, R., Plancot, B., Ropitaux, M., Carreras, A., Leprince, J., Boulogne, I., Follet-Gueye, M.L., Popper, Z.A., Driouich, A., and Vicre, M. (2018). Cell wall extensins in root-microbe interactions and root secretions. J. Exp. Bot. *69*, 4235-4247.
31. Bradley, D.J., Kjellbom, P., and Lamb, C.J. (1992). Elicitor and wound-induced oxidative cross-linking of a proline-rich plant-cell wall protein: a novel, rapid defense response. Cell *70*, 21-30.
32. Liu, X., Wolfe, R., Welch, L.R., Domozych, D.S., Popper, Z.A., and Showalter, A.M. (2016). Bioinformatic identification and analysis of extensins in the plant kingdom. PLoS One *11*.
33. Bai, L., Zhang, G.Z., Zhou, Y., Zhang, Z.P., Wang, W., Du, Y.Y., Wu, Z.Y., and Song, C.P. (2009). Plasma membrane-associated proline-rich extensin-like receptor kinase 4, a novel regulator of Ca2+ signalling, is required for abscisic acid responses in *Arabidopsis thaliana*. Plant J. *60*, 314-327.
34. Deeks, M.J., Fendrych, M., Smertenko, A., Bell, K.S., Oparka, K., Cvrckova, F., Zarsky, V., and Hussey, P.J. (2010). The plant formin AtFH4 interacts with both actin and microtubules, and contains a newly identified microtubule-binding domain. J. Cell Sci. *123*, 1209-1215.
35. Ingouff, M., Fitz Gerald, J.N., Guerin, C., Robert, H., Sorensen, M.B., Van Damme, D., Geelen, D., Blanchoin, L., and Berger, F. (2005). Plant formin AtFH5 is an evolutionarily conserved actin nucleator involved in cytokinesis. Nat. Cell. Biol. *7*, 374-380.
36. Cheung, A.Y., and Wu, H.M. (2004). Overexpression of an Arabidopsis formin stimulates supernumerary actin cable formation from pollen tube cell membrane. Plant Cell *16*, 257-269.
37. Hohmann, U., Lau, K., and Hothorn, M. (2017). The structural basis of ligand perception and signal activation by receptor kinases. Annu. Rev. Plant Biol. *68*, 109-137.
38. Moussu, S., Broyart, C., Santos-Fernandez, G., Augustin, S., Wehrle, S., Grossniklaus, U., and Santiago, J. (2019). Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth. BioRxiv, http://dx.doi.org/10.1101/695874.
39. Ringli, C. (2010). The hydroxyproline-rich glycoprotein domain of the Arabidopsis LRX1 requires Tyr for function but not for insolubilization in the cell wall. Plant J. *63*, 662-669.

40. Qi, X.Y., Behrens, B.X., West, P.R., and Mort, A.J. (1995). Solubilization and partial characterization of extensin fragments from cell walls of cotton suspension-cultures - evidence for a covalent cross-link between extensin and pectin. Plant Physiol. *108*, 1691-1701.
41. Chen, Y., Dong, W., Tan, L., Held, M.A., and Kieliszewski, M.J. (2015). Arabinosylation plays a crucial role in extensin cross-linking in vitro. Biochemical Insights *8*, 1-13.
42. Baumberger, N., Doesseger, B., Guyot, R., Diet, A., Parsons, R.L., Clark, M.A., Simmons, M.P., Bedinger, P., Goff, S.A., Ringli, C., et al. (2003a). Whole-genome comparison of leucine-rich repeat extensins in Arabidopsis and rice: a conserved family of cell wall proteins form a vegetative and a reproductive clade. Plant Physiol. *131*, 1313-1326.
43. Fabrice, T., Vogler, H., Draeger, C., Munglani, G., Gupta, S., Herger, A.G., Knox, P., Grossniklaus, U., and Ringli, C. (2018). LRX proteins play a crucial role in pollen grain and pollen tube cell wall development. Plant Physiol. *176*, 1981-1992.
44. Cross, L.L., Ebeed, H.T., and Baker, A. (2016). Peroxisome biogenesis, protein targeting mechanisms and PEX gene functions in plants. Biochim. Biophys. Acta-Mol. Cell Res. *1863*, 850-862.
45. Baumberger, N., Ringli, C., and Keller, B. (2001). The chimeric leucine-rich repeat/extensin cell wall protein LRX1 is required for root hair morphogenesis in *Arabidopsis thaliana*. Genes Dev. *15*, 1128-1139.
46. Baumberger, N., Steiner, M., Ryser, U., Keller, B., and Ringli, C. (2003b). Synergistic interaction of the two paralogous Arabidopsis genes *LRX1* and *LRX2* in cell wall formation during root hair development. Plant J. *35*, 71-81.
47. Lewis, D.R., Olex, A.L., Lundy, S.R., Turkett, W.H., Fetrow, J.S., and Muday, G.K. (2013). A kinetic analysis of the auxin transcriptome reveals cell wall remodeling proteins that modulate lateral root development in Arabidopsis. Plant Cell *25*, 3329-3346.
48. Draeger, C., Fabrice, T.N., Gineau, E., Mouille, G., Kuhn, B.M., Moller, I., Abdou, M.-T., Frey, B., Pauly, M., Bacic, A., et al. (2015). Arabidopsis leucine-rich repeat extensin (LRX) proteins modify cell wall composition and influence plant growth. BMC Plant Biol. *15*, doi.org/10.1186/s12870-12015-10548-12878.
49. Dünser, K., Gupta, S., Herger, A., Feraru, M.I., Ringli, C., and Kleine-Vehn, J. (2019). Extracellular matrix sensing by FERONIA and Leucine-Rich Repeat Extensins controls vacuolar expansion during cellular elongation in *Arabidopsis thaliana*. EMBO J., 10.15252/embj.2018100353.
50. Zhao, C.Z., Zayed, O., Yu, Z.P., Jiang, W., Zhu, P.P., Hsu, C.C., Zhang, L.R., Tao, W.A., Lozano-Duran, R., and Zhu, J.K. (2018). Leucine-rich repeat extensin proteins regulate plant salt tolerance in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. *115*, 13123-13128.
51. Mollet, J.-C., Leroux, C., Dardelle, F., and Lehner, A. (2013). Cell wall composition, biosynthesis and remodeling during pollen tube growth. Plants *2*, 107-147.
52. Sede, A.R., Borassi, C., Wengier, D.L., Mecchia, M.A., Estevez, J.M., and Muschietti, J.P. (2018). Arabidopsis pollen extensins LRX are required for cell wall integrity during pollen tube growth. Febs Lett. *592*, 233-243.
53. Wang, X.X., Wang, K.Y., Yin, G.M., Liu, X.Y., Liu, M., Cao, N.N., Duan, Y.Z., Gao, H., Wang, W.L., Ge, W.N., et al. (2018). Pollen-expressed leucine-rich repeat extensins are essential for pollen germination and growth. Plant Physiol. *176*, 1993-2006.
54. Mecchia, M.A., Santos-Fernandez, G., Duss, N.N., Somoza, S.C., Boisson-Dernier, A., Gagliardini, V., Martinez-Bernardini, A., Fabrice, T.N., Ringli, C., Muschietti, J.P., et al. (2017). RALF4/19 peptides interact with LRX proteins to control pollen tube growth in Arabidopsis. Science *358*, 1600-1603.
55. Covey, P.A., Subbaiah, C.C., Parsons, R.L., Pearce, G., Lay, F.T., Anderson, M.A., Ryan, C.A., and Bedinger, P.A. (2010). A pollen-specific RALF from tomato that regulates pollen tube elongation. Plant Physiol. *153*, 703-715.
56. Murphy, E., and De Smet, I. (2014). Understanding the RALF family: a tale of many species. Trends Plant Sci. *19*, 664-671.

57. Campbell, L., and Turner, S.R. (2017). Comprehensive analysis of RALF proteins in green plants suggests there are two distinct functional groups. Front. Plant Sci. *8*.
58. Pearce, G., Moura, D.S., Stratmann, J., and Ryan, C.A. (2001). RALF, a 5-kDa ubiquitous polypeptide in plants, arrests root growth and development. Proc. Natl. Acad. Sci. U.S.A. *98*, 12843-12847.
59. Stegmann, M., Monaghan, J., Smakowska-Luzan, E., Rovenich, H., Lehner, A., Holton, N., Belkhadir, Y., and Zipfel, C. (2017). The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. Science *355*, 287-289.
60. Ge, Z.X., Bergonci, T., Zhao, Y.L., Zou, Y.J., Du, S., Liu, M.C., Luo, X.J., Ruan, H., Garcia-Valencia, L.E., Zhong, S., et al. (2017). Arabidopsis pollen tube integrity and sperm release are regulated by RALF-mediated signaling. Science *358*, 1596-1599.
61. Rayle, D., and Cleland, R. (1970). Enhancement of wall loosening and elongation by acid solutions. Plant Physiol. *46*, 250-253.
62. Haruta, M., Sabat, G., Stecker, K., Minkoff, B.B., and Sussman, M.R. (2014). A peptide hormone and its receptor protein kinase regulate plant cell expansion. Science *343*, 408-411.
63. Haruta, M., Monshausen, G., Gilroy, S., and Sussman, M.R. (2008). A cytoplasmic Ca2+ functional assay for identifying and purifying endogenous cell signaling peptides in Arabidopsis seedlings: Identification of AtRALF1 peptide. Biochemistry *47*, 6311-6321.
64. Bergonci, T., Ribeiro, B., Ceciliato, P.H., Guerrero-Abad, J.C., Silva-Filho, M.C., and Moura, D.S. (2014). Arabidopsis thaliana RALF1 opposes brassinosteroid effects on root cell elongation and lateral root formation. J. Exp. Bot. *65*, 2219-2230.
65. Gonneau, M., Desprez, T., Martin, M., Doblas, V.G., Bacete, L., Miart, F., Sormani, R., Hematy, K., Renou, J., Landrein, B., et al. (2018). Receptor kinase THESEUS1 is a rapid alkalinization factor 34 receptor in Arabidopsis. Curr. Biol. *28*, 2452-2458.
66. Moussu, S., Augustin, S., Roman, A.O., Broyart, C., and Santiago, J. (2018). Crystal structures of two tandem malectin-like receptor kinases involved in plant reproduction. Acta Crystallogr. D *74*, 671-680.
67. Merz, D., Richter, J., Gonneau, M., Sanchez-Rodriguez, C., Eder, T., Sormani, R., Martin, M., Hematy, K., Hofte, H., and Hauser, M.T. (2017). T-DNA alleles of the receptor kinase *THESEUS1* with opposing effects on cell wall integrity signaling. J. Exp. Bot. *68*, 4583-4593.
68. Escobar-Restrepo, J.M., Huck, N., Kessler, S., Gagliardini, V., Gheyselinck, J., Yang, W.C., and Grossniklaus, U. (2007). The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception. Science *317*, 656-660.
69. Feng, W., Kita, D., Peaucelle, A., Cartwright, H.N., Doan, V., Duan, Q.H., Liu, M.C., Maman, J., Steinhorst, L., Schmitz-Thom, I., et al. (2018). The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca2+ signaling. Curr. Biol. *28*, 666-675.
70. Ngo, Q.A., Vogler, H., Lituiev, D.S., Nestorova, A., and Grossniklaus, U. (2014). A calcium dialog mediated by the FERONIA signal transduction pathway controls plant sperm delivery. Dev. Cell *29*, 491-500.
71. Shih, H.W., Miller, N.D., Dai, C., Spalding, E.P., and Monshausen, G.B. (2014). The receptor-like kinase FERONIA is required for mechanical signal transduction in Arabidopsis seedlings. Curr. Biol. *24*, 1887-1892.
72. Boisson-Dernier, A., Lituiev, D.S., Nestorova, A., Franck, C.M., Thirugnanarajah, S., and Grossniklaus, U. (2013). ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases. Plos Biology *11*.
73. Kwon, T., Sparks, J.A., Liao, F.Q., and Blancaflor, E.B. (2018). ERULUS is a plasma membrane-localized receptor-like kinase that specifies root hair growth by maintaining tip-focused cytoplasmic calcium oscillations. Plant Cell *30*, 1173-1177.
74. Schoenaers, S., Balcerowicz, D., Breen, G., Hill, K., Zdanio, M., Mouille, G., Holman, T.J., Oh, J., Wilson, M.H., Nikonorova, N., et al. (2018). The auxin-regulated *Cr*RLK1L kinase ERULUS controls cell wall composition during root hair tip growth. Curr. Biol. *28*, 722-+.

75. Dünser, K., Gupta, S., Ringli, C., and Kleine-Vehn, J. (2017). LRX- and FER-dependent extracellular sensing coordinates vacuolar size for cytosol homeostasis. bioRxiv, 231043.
76. Li, C., Yeh, F.L., Cheung, A.Y., Duan, Q., Kita, D., Liu, M.C., Maman, J., Luu, E.J., Wu, B.W., Gates, L., et al. (2015). Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in Arabidopsis. Elife *4*.
77. Duan, Q.H., Kita, D., Li, C., Cheung, A.Y., and Wu, H.M. (2010). FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. Proc. Natl. Acad. Sci. U.S.A. *107*, 17821-17826.
78. Fry, S.C. (1986). Cross-linking of matrix polymers in the growing cell-walls of angiosperms. Annu. Rev. Plant Physiol. Plant Molec. Biol. *37*, 165-186.
79. Diet, A., Link, B., Seifert, G.J., Schellenberg, B., Wagner, U., Pauly, M., Reiter, W.D., and Ringli, C. (2006). The Arabidopsis root hair cell wall formation mutant *lrx1* is suppressed by mutations in the *RHM1* gene encoding a UDP-L-rhamnose synthase. Plant Cell *18*, 1630-1641.
80. Leiber, R.M., John, F., Verhertbruggen, Y., Diet, A., Knox, J.P., and Ringli, C. (2010). The TOR pathway modulates the structure of cell walls in Arabidopsis. Plant Cell *22*, 1898-1908.
81. Schaufelberger, M., Galbier, F., Herger, A., de Brito Francisco, R., Roffler, S., Clement, G., Diet, A., Hortensteiner, S., Wicker, T., and Ringli, C. (2019). Mutations in the Arabidopsis *ROL17/isopropylmalate synthase 1* locus alter amino acid content, modify the TOR network, and suppress the root hair cell development mutant *lrx1*. J. Exp. Bot. *70*, 2313-2323.
82. Field, B., Cardon, G., Traka, M., Botterman, J., Vancanneyt, G., and Mithen, R. (2004). Glucosinolate and amino acid biosynthesis in Arabidopsis. Plant Physiol. *135*, 828-839.
83. de Kraker, J.W., Luck, K., Textor, S., Tokuhisa, J.G., and Gershenzon, J. (2007). Two Arabidopsis genes (*IPMS1* and *IPMS2*) encode isopropylmalate synthase, the branchpoint step in the biosynthesis of leucine. Plant Physiol. *143*, 970-986.
84. Loewith, R., and Hall, M.N. (2011). Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics *189*, 1177-1201.
85. John, F., Philipp, M., Leiber, R.M., Errafi, S., and Ringli, C. (2014). Ubiquitin-related modifiers of *Arabidopsis thaliana* influence root development. PLoS One *9*, 22.
86. Philipp, M., John, F., and Ringli, C. (2014). The cytosolic thiouridylase CTU2 of *Arabidopsis thaliana* is essential for posttranscriptional thiolation of tRNAs and influences root development. BMC Plant Biol. *14*.
87. Laxman, S., Sutter, B.M., Wu, X., Kumar, S., Guo, X., Trudgian, D.C., Mirzaei, H., and Tu, B.P. (2013). Sulfuramino acids regulate translational capacity and metabolic homeostasis through modulation of tRNA thiolation. Cell *154*, 416-429.
88. Gonzalez, A., and Hall, M.N. (2017). Nutrient sensing and TOR signaling in yeast and mammals. EMBO J. *36*, 397-408.
89. Laplante, M., and Sabatini, D.M. (2012). mTOR signaling in growth control and disease. Cell *149*, 274-293.
90. Schepetilnikov, M., Makarian, J., Srour, O., Geldreich, A., Yang, Z., Chicher, J., Hammann, P., and Ryabova, L.A. (2017). GTPase ROP2 binds and promotes activation of target of rapamycin, TOR, in response to auxin. EMBO J. *36*, 886-903.

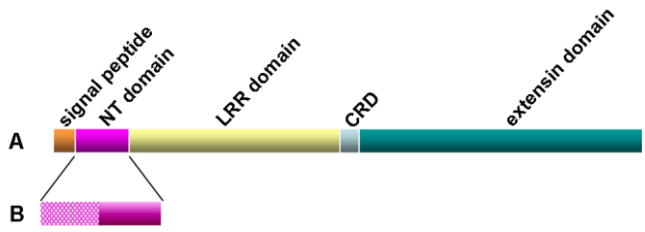

Figure 1. Structure of LRX proteins.

A LRX proteins contain a signal peptide for protein export (orange), an N-termial domain (NT-domain, purple), an LRR domains with 10 full leucine-rich repeats (yellow), a Cys-rich linker region (CRD, grey) and a C-terminal extensin domain.

B The NT-domain consists of a first half that is highly variable or missing among LRXs (dotted) and a well conserved second half.

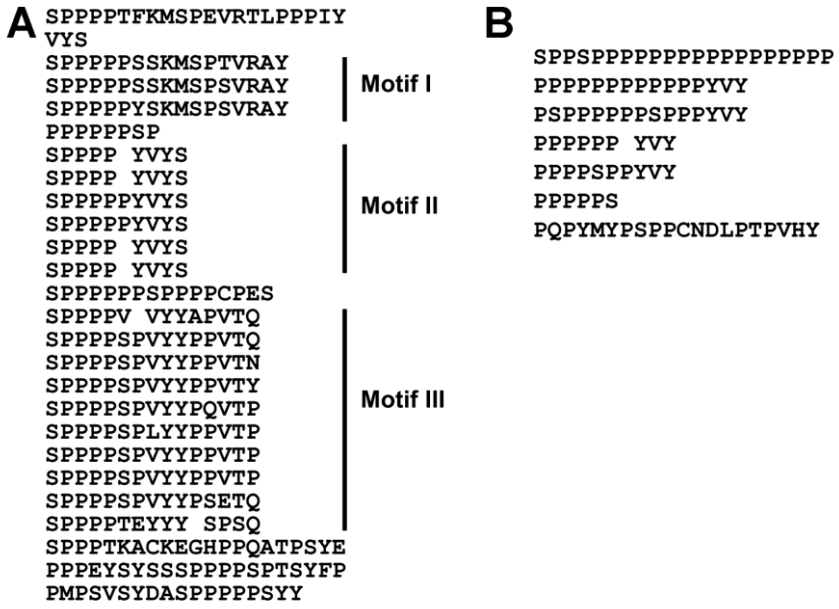

Figure 2. Variability in the extensin domain of LRX1 and LRX6.

A The LRX1 extensin domain consists of several distinct sequence motifs, all containing the Ser-Pro_{2-n} repeat characteristic for extensins. Pro residues are posttranslationally hydroxylated to Hydroxproline.

B The much smaller extensin domain of LRX6.

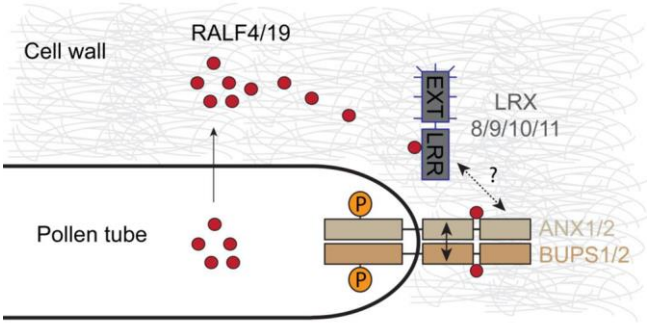

Figure 3. LRX proteins and RALF4/19 regulate pollen tube growth.

Secreted RALF4 and RALF19 peptides bind to pollen-expressed LRXs as well as the CrRLK1Ls BUPS1/2 and ANX1/2. These associations prevent precocious pollen tube rupture by maintaining cell wall integrity. Future research is needed to clarify whether LRXs additionally interact with BUPS1/2 and ANX1/2, which moreover can form heteromeric complexes.

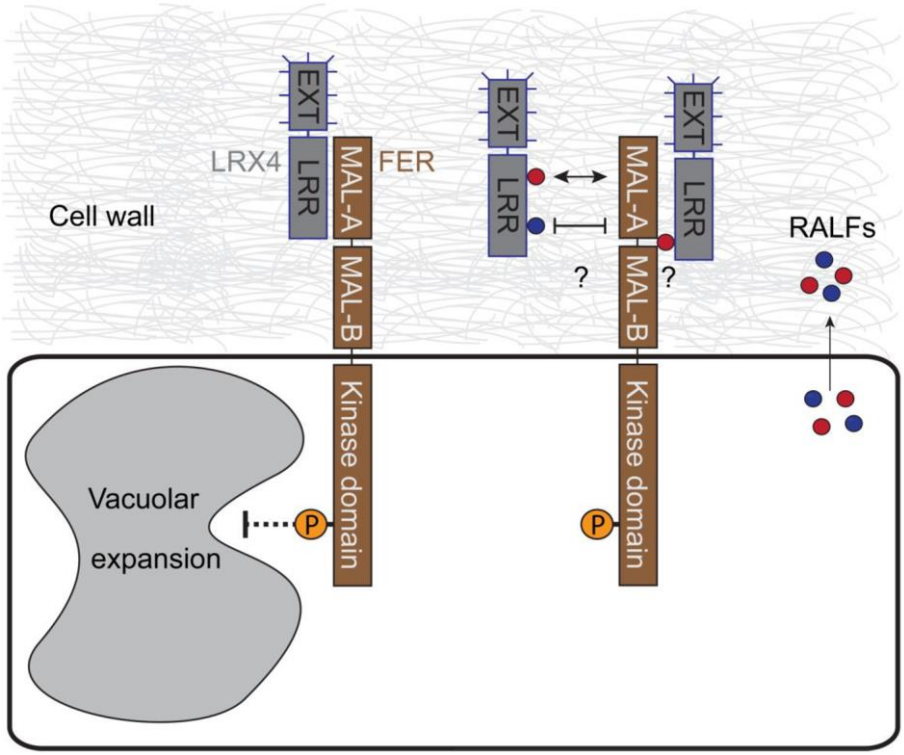

Figure 4. LRX- and FER-dependent cell wall sensing impacts vacuolar expansion during cell elongation.

The extensin domain of LRX is anchored in the extracellular matrix, perceiving changes in the cell wall, while the LRR domain interacts with FER to transduce the signal and trigger inflation of the vacuole. The kinase domain of FER is required for this process. RALFs bind to FER as well as LRX4, hence it will be interesting to see whether distinct RALFs are capable of promoting or preventing the interaction of LRX4 with FER and potential additional components. However, LRX/FER function in cell wall sensing might be independent of their role in RALF signaling.